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Fetal lung maturity assessment by a modified A_{650} determination

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1 Introduction

The biophysical characterization of amniotic fluid by its absorbance at 650 nm is widely used as a method for assessing fetal lung maturity. Centrifugation of amniotic fluid to remove cells and debris is common to most methodologies but recommended centrifugation conditions vary widely (250 × g for 5 minutes [6] to 12,000 × g for 10 minutes [2]), making comparisons of absorbance data from various laboratories difficult. In several studies centrifugation at 2,000 × g for 10 minutes has been used [3, 9, 10]. However, other investigators have shown that centrifugation under those conditions can result in the sedimentation of a significant portion of the phospholipid-containing lamellar bodies [5–8]. This has led to the recommendation of centrifugation at 250 × g for 5 minutes [6].

The present study was undertaken to evaluate the usefulness of a differential centrifugation procedure and to estimate the possible contribution of non-sedimentable material to absorbance measurements as generally carried out.

The absorbance data obtained by this procedure are compared to the L/S ratio and absorbance after centrifugation at 2,000 × g as described by SBARRA et al. [9] as predictors of neonatal pulmonary performance and the occurrence of hyaline membrane disease when delivery occurred within 3 days of sample collection.

Curriculum vitae

MICHAEL F. KOSZALKA *Jr., M. D., graduated from the University of Minnesota School of Medicine in 1972. Residency in Obstetrics and Gynecology was completed in 1976 at St. Paul-Ramsey Hospital. Certification by the American Board in 1979, preceded fellowship in Maternal-Fetal Medicine at Denver General Hospital from 1979 to 1981. He returned to St. Paul-Ramsey in 1981 as Perinatal Center Obstetrical Division Co-Director, where he is currently in practice.*

2 Methodology

2.1 Sample population

Samples of amniotic fluid were obtained transabdominally or as vaginal aspirates, for clinically indicated diagnostic testing. Patient informed consent was obtained in accordance with the policies of the Institutional Review Board at St. Paul-Ramsey Medical Center. Samples were stored at 4 °C and analyzed within 48 hours of acquisition. In preliminary experiments we established that, in agreement with SHUGAR [11], storage for this interval did not affect absorbance values.

Data were obtained on fluids from the total sample population (N = 281) and from a corrected population (N = 172) in which specimens from pregnancies complicated by Rh isoimmunization (N = 5), diabetes (N = 36), and premature rupture of membranes (N = 31), and those with blood, and/or meconium contaminants (N = 37) were removed.

Gestational age at sampling was calculated from the date of sampling and the gestational age determined by pediatric newborn exam using BALLARD criteria [1]. Gestational ages ranged from 25 to 43 weeks.

2.2 Absorbance measurements

Samples were divided into two parts. One part was used for L/S quantitation and the other for absorbance measurements. This latter portion was initially centrifuged at 250 × g for 5 minutes, to minimize loss of lamellar bodies [4, 5], and the absorbance at 650 nm (A_{250}) measured with a Gilford Model 240 spectrophotometer (1 cm light path) with air used as the blank. With some samples, in order to obtain absorbance data suitable for comparison with studies from other laboratories in which only $A_{2,000}$ was measured, the supernatant fluid from the 250 × g spin was centrifuged at 2,000 × g for 10 minutes and absorbance measured ($A_{2,000}$). To assess if prior centrifugation at 250 × g affected absorbance after centrifugation at 2,000 × g, 13 samples were divided into two parts and these subjected either to a single centrifugation at 2,000 × g or the two-step centrifugation. Mean $A_{2,000}$ values were 0.453, range 0.095 to 1.233, (two-step centrifugation) and 0.448, range 0.096 to 1.139 (single centrifugation). The supernatant from the 2,000 × g spin was then centrifuged at 10,000 × g for 20 minutes to allow for the quantitation of absorbance at 650 nm ($A_{10,000}$) after removal of lamellar body material [8].

2.3 L/S Ratio

The procedure of TSAI and associates [12, 13], the method adopted by St. Paul-Ramsey Medical Center and the University of Minnesota Hospitals, was used to quantitate the L/S ratio on the remaining aliquot of the initial sample. An L/S ratio greater than or equal to 2.1 was called mature.

2.4 Data analyses

Values for absorbance after the 250 × g (A_{250}) and 10,000 × g ($A_{10,000}$) centrifugations were used to generate two new parameters: ΔA ($A_{250} - A_{10,000}$), to focus on absorbance due to material sedimentable between 250 and 10,000 × g, principally lamellar bodies [5, 8], and %A ($\Delta A/A_{250} \times 100$) to express the sedimentable material as a function of the total absorbing material present and thus minimize dilution effects. To eliminate the contribution of the non-sedimentable or background absorbance and decrease effects of dilution, ΔA and %A were used in combination with A_{250} to create an absorbance battery (A_{Batt}). On the basis of data from a pilot series of 25 samples, A_{250} was designated as mature if greater than or equal to .350. ΔA was called mature if greater than or equal to .250. %A was considered mature if greater than or equal to 75%. The A_{Batt} was considered immature if any one of the parameters was immature. $A_{2,000}$ of greater than or equal to 0.150 was called mature. Sensitivity, specificity and predictive value were calculated as described by CREASY and SIMON [4]. These calculations for A_{Batt} are shown in table I.

Table I. Binary table used to calculate specificity, sensitivity and predictive value for A_{Batt}.

	HMD	No HMD
A_{Batt} (immature)	a	c
A_{Batt} (mature)	b	d

Sensitivity $= \frac{a}{a+b} = \frac{6}{6+4}$; specificity $= \frac{d}{c+d} = \frac{114}{114+32}$; positive predictive value $= \frac{a}{a+c} = \frac{6}{6+32}$; negative predictive value $= \frac{d}{b+d} = \frac{114}{4+114}$.

3 Results

Median values and ranges of values for each absorbance parameter increased with gestational age. This is illustrated in figure 1, which shows median values of A_{250} (N = 281), $A_{2,000}$ (N = 149), and $A_{10,000}$ (N = 281) for samples from the total population, and of A_{250} (N = 172) for samples from the corrected population plotted against gestational age at sampling. The ranges of values for A_{250}, $A_{2,000}$ and $A_{10,000}$ were 0.095 to 3.00, 0.044 to 1.70, and 0.036 to 1.12 respectively for the total population. These were similar to the example given for A_{250} of the total population where the 90th percentile is illustrated when sample numbers exceeded ten at 32, 34, 36, 38, and 40 weeks of gestation. Figures 2 and 3 are scatter-diagrams showing the increase in ΔA and %A with gestation for samples from the total population. The corrected population (not illustrated) gave similar results.

A second objective of this study was to relate maturity estimates based on L/S and absorbance parameters and complications in the newborn due to occurrence of hyaline membrane disease (HMD). Newborns delivered within 72 hours of sampling from 157 patients were reviewed by a neonatologist without knowledge of L/S values or absorbance data. A diagnosis of HMD required the presence of the following: hypoxemia, radiologic evidence of atelectasis, and a clinical course compatible with the syndrome in the absence of sepsis. Tab. II shows that the L/S ratio had a higher specificity (94.6%) and sensitivity (87.5%) than either the

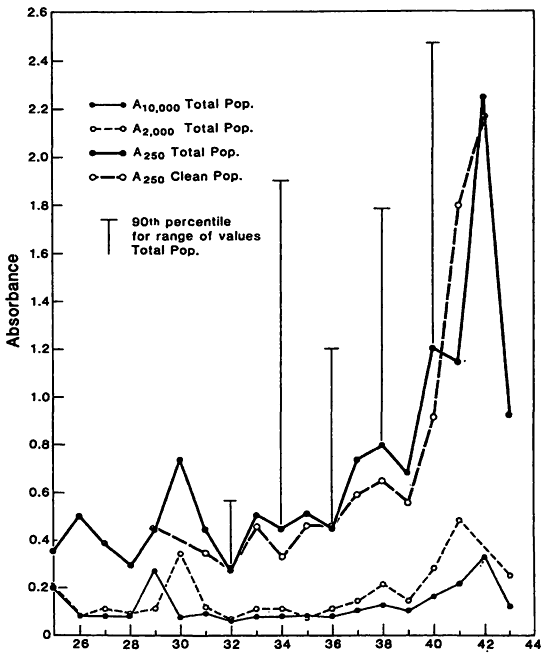

Figure 1. Median absorbance values for A_{250}, $A_{2,000}$ and $A_{10,000}$ and range of values for A_{250} according to gestational age.

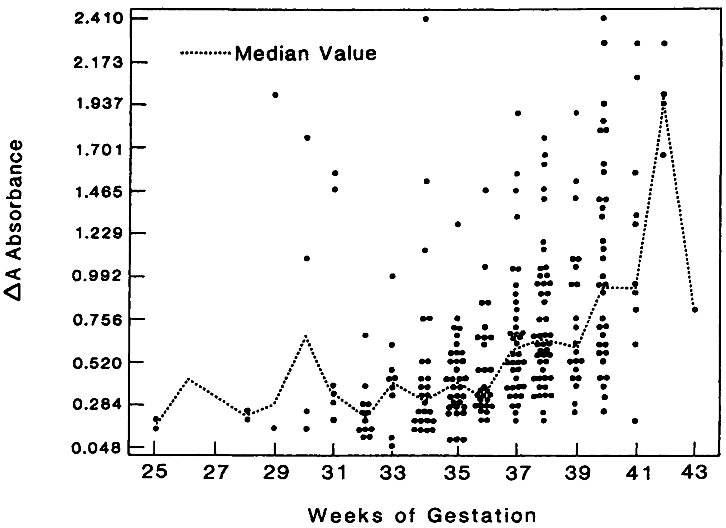

Figure 2. Scatterdiagram showing ΔA in relation to gestational age.

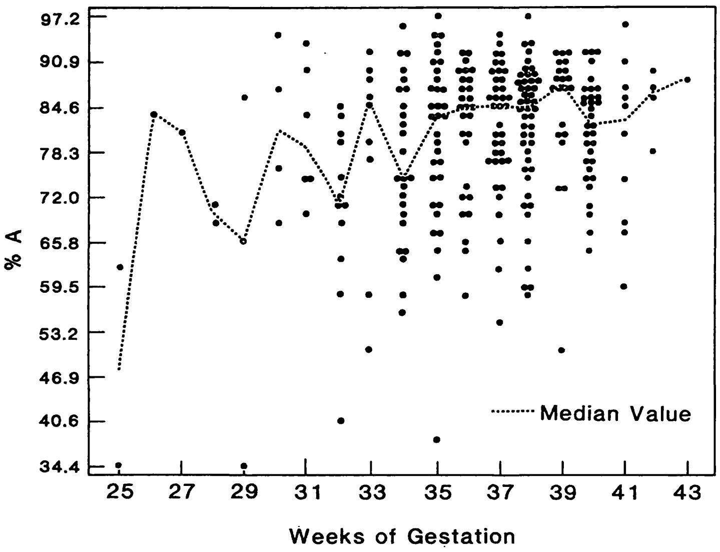

Figure 3. Scatterdiagram showing distribution of %A in relation to gestational age.

A_{Batt} or $A_{2,000}$ methods. The predictive value of a negative test was high for all three tests, 99.2%, 96.6%, and 96.2% for L/S, A_{Batt} and $A_{2,000}$. The predictive value of a positive test (presence of HMD) for the L/S ratio (50%) was better than either absorbance method. Analysis of the components of A_{Batt} individually or in other combination failed to improve specificity or sensitivity.

All false negative predictions of lung immaturity on the basis of absorbance data occurred in samples contaminated with blood or meconium. Analysis of the corrected population (not illustrated) excluded samples from pregnancies complicated by PROM, diabetes, isoimmunization, and fluids with contaminants and resulted in elimination of all false positive predictions of lung maturity with all three tests.

4 Conclusion

As shown in figure 1, A_{250}, $A_{2,000}$ and $A_{10,000}$ increased with gestational age. A_{250} was greater than $A_{2,000}$ at each point, consistent with the loss of lamellar body material after centrifugation, as previously reported [6]. The increases in $A_{2,000}$ and $A_{10,000}$ appear to represent increases in lamellar bodies as well as non-sedimentable material. That this latter component, as estimated by $A_{10,000}$, can contribute significantly to $A_{2,000}$ has not been considered previously. Because of the range of A_{250} values at each gestational age, we proposed that the frequency of incorrect predictions of lung maturity (false negative predictions of lung immaturity), the most adverse possibility clinically, could be minimized by interpreting A_{250} in combination with ΔA and %A which also increase during gestation. As shown in table II, absorbance methods had similar sensitivities and specificities. Thus, in our hands, A_{Batt} and $A_{2,000}$ were equally effective in predicting lung maturity. A significant aspect of this study, however, is that it suggests that the basis for this effectiveness differs in the two cases. The increases in A_{250}, ΔA, and %A with gestational age are consistent with the known increases in amniotic fluid content of lamellar bodies. Thus their ability to predict lung maturity appears to be directly related to amniotic fluid lecithin content [5, 8]. In contrast, the fact that a significant fraction of lamellar bodies is lost on centrifugation at 2,000 × g for 10 minutes [5, 6, 8], when considered with the increases in $A_{10,000}$ during gestation (figure 1) suggests that the predictive value of $A_{2,000}$ derives only in part from the presence of lamellar bodies and is due, also, to non-lamellar body, non-sedimenting material.

That each of the false negative predictions of immaturity, on the basis of absorbance, occurred with bloody samples suggests in agreement with other reports [9], that absorbance analysis be restricted to uncontaminated specimens.

Table II. Table shows specificity, sensitivity, and predictive value of L/S ratio, A_{Batt} and $A_{2,000}$ and the presence or absence of HMD.

	Hyaline Membrane Disease present	absent	Specificity/Sensitivity	Predictive Value + test	+ test
L/S immature	7	7	94.6%/87.5%	50%	99.2%
mature	1	125			
A_{Batt} immature	6	32	78.1%/60%	16%	96.6%
mature	4	114			
$A_{2,000}$ immature	4	31	62.2%/66%	11%	96.2%
mature	2	31			

Summary

This paper presents an evaluation of a modified absorbance method for estimating fetal lung maturity. Absorbance at 650 nm in combination with a two-step centrifugation procedure was used in an attempt to focus more directly on lamellar bodies and evaluate the contribution of residual absorbance due to non-lamellar body materials. Absorbance values after centrifugation at 250 × g for 5 minutes (A_{250}) and 10,000 × g for 20 minutes ($A_{10,000}$) were taken as estimates of total absorbance due to lamellar bodies plus non-lamellar body material and that due to non-sedimentable, non-lamellar body material respectively. These values were used to generate two new parameters: ΔA ($A_{250} - A_{10,000}$), to better estimate absorbance due to lamellar bodies, and %A (ΔA/A_{250} × 100), to express lamellar body absorbance in terms of total observable absorbance and thereby minimize effects of dilution. The three parameters (A_{250}, ΔA, %A) were used in combination to create a battery (A_{Batt}) of absorbance values for each fluid sample. Absorbance after centrifugation at 2,000 × g for 10 minutes ($A_{2,000}$), a widely used standard method, was also evaluated for purposes of comparison. A_{250} was designated as mature if greater than or equal to 0.350, ΔA was called mature if greater than or equal to 0.250, and %A was considered mature if greater than or equal to 75%. If any of the parameters was immature, A_{Batt} was called immature. The range of values for A_{250}, $A_{2,000}$, and $A_{10,000}$, (figure 1) increased with gestational age in the total population as well as the corrected population (excluding amniotic fluid contaminants, and pregnancies with isoimmunization or diabetes). Median values for A_{250} increased more than $A_{2,000}$ with advancing gestational age. Non-sedimentable, residual absorbance ($A_{10,000}$) also increased with advancing gestational age, however, its relative contribution to A_{250} is less than to $A_{2,000}$ beyond 36 weeks gestation. The second portion of the study was to evaluate the relationship between L/S values, absorbance parameters and newborn complications when delivery occurred within three days of amniotic fluid sampling. The non-occurrence of HMD was most accurately predicted by the L/S ratio in the total population (Tab. II), and equally by all three methods in the corrected population. False predictions of maturity on the basis of absorbance data were eliminated when the sample population was restricted to uncontaminated specimens obtained in the absence of maternal diabetes, isoimmunization and premature rupture of membranes.

This study demonstrates that the non-occurrence of HMD can be predicted by either the L/S ratio, the A_{Batt} or the $A_{2,000}$ method. The data suggest that the usefulness of A_{Batt} derives principally from amniotic fluid lamellar bodies, while both lamellar bodies and non-sedimentable material contribute to $A_{2,000}$.

Keywords: Absorbance 650 nm, amniotic fluid analysis, fetal lung maturity.

Zusammenfassung

Bestimmung der fetalen Lungenreife mit einer modifizierten Absorptionsmessung bei 650 nm

In der vorliegenden Arbeit berichten wir über eine modifizierte Absorptionsmessung zur Abschätzung der fetalen Lungenreife. Die Bestimmung der Absorption bei 650 nm in Kombination mit zweimaligem Zentrifugieren bei unterschiedlichen Geschwindigkeiten wurde eingesetzt, um direkt die durch die Lamellärkörperchen erzeugte Absorption und den Anteil der Restabsorption durch nichtlamelläre Bestandteile zu erfassen. Der Extinktionswert nach fünfminütiger Zentrifugation bei 250 × g (A_{250}) zeigt die Absorption durch Lamellärkörperchen *plus* nichtlamelläre Bestandteile an; der Wert nach zwanzigminütiger Zentrifugation bei 10 000 × g (A_{10000}) erfaßt ausschließlich die Absorption durch nicht sedimentierbare, nichtlamelläre Bestandteile. Über diese Werte lassen sich zwei neue Parameter beschreiben: A ($A_{250} - A_{10000}$) als Schätzwert der Absorption durch Lamellärkörperchen und %A (ΔA/A_{250} × 100) als Ausdruck für den Anteil der durch Lamellärkörperchen bedingten Absorption von der Gesamtabsorption. Auf diese Weise läßt sich der Einfluß durch die Verdünnung minimieren. Diese 3 Parameter (A_{250}, ΔA, %A) wurden in Beziehung zueinander gesetzt und so ein Kombinationswert für die Absorption (A_{Batt}) geschaffen, der für jede Probe bestimmt wurde. Zum Vergleich wurden die Absorptionswerte nach zehnminütiger Zentrifugation bei 2 000 × g (A_{2000}) gemessen; dieses Vorgehen gilt als Standardmethode. Bei einem A_{250}-Wert ≥ 0.350, einem ΔA-Wert ≥ 0.250 und einem %A-Wert ≥ 75% wurde eine positive Lungenreife vorausgesagt. Lag einer dieser Parameter unter den genannten Grenzen, wurde über A_{Batt} eine negative Lungenreife vorausgesagt. Sowohl im Gesamtkollektiv wie auch in der korrigierten Gruppe (Ausschluß von kontaminierten Fruchtwasserproben und Schwangerschaften mit Sensibilisierung oder Diabetes) stiegen die A_{250}-, A_{2000}- und A_{10000}-Werte mit dem Gestationsalter an (Abb. 1). Die Medianwerte für A_{250} zeigten mit fortschreitendem Schwangerschaftsalter einen stärkeren Anstieg als die für A_{2000}. Die auf nicht sedimentierbare Bestandteile zurückgehende Restabsorption (A_{10000}) stieg ebenfalls mit fortschreitendem Gestationsalter an. Unterhalb der 36. Woche ist deren Anteil am A_{250}-Wert jedoch geringer als am A_{2000}-Wert. Der zweite Teil der Studie galt den Beziehungen zwischen L/S-Werten, Absorptionsparametern und den Komplikationen bei den Neugeborenen, wobei die Geburt maximal 3 Tage nach der Fruchtwasserentnahme erfolgte. In der Gesamtgruppe wurde die Lungenreife durch die L/S-Ratio am genauesten vorausgesagt, während sich in der korrigierten Gruppe die 3 Methoden nicht unterschieden. Die mit den Absorptionswerten erhobenen falsch positiven Ergebnisse konnten dann eliminiert werden, wenn nur unkontaminierte Fruchtwasserproben von Schwangeren ohne Diabetes, Rhesussensibilisierung oder vorzeitigem Blasensprung berücksichtigt wurden. Die Untersuchung zeigt, daß die Lungenreife sowohl durch die L/S-Ratio wie auch den A_{Batt}-Wert und die A_{2000}-Methode vorausgesagt werden kann. Der A_{Batt}-Wert ist deshalb brauchbar, weil hier der Anteil der Lamellärkörperchen gesondert berücksichtigt wird, während bei der A_{2000}-Methode sowohl Lamellärkörperchen wie auch nicht sedimentierbare Bestandteile erfaßt werden.

Schlüsselwörter: Absorption bei 650 nm, fetale Lungenreife, Fruchtwasseruntersuchung.

Résumé

Estimation de la maturité pulmonaire fœtale par la determination A_{650} modifiée

Cet article présente l'évaluation d'une méthode d'absorption modifiée pour l'estimation de la maturité pulmonaire fœtale. On a utilisé l'absorption à 650 nm associé à un procédé de centrifugation à deux niveaux dans l'optique de se centrer plus directement sur les corps lamellaires et d'évaluer la contribution de l'absorption résiduelle due au matériel autre que les corps lamellaires. On a considéré que les valeurs d'absorption après centrifugation à 250 × g pendant 5 minutes (A_{250}) et à 10 000 × g pendant 20 minutes représentaient respectivement l'estimation de l'absorption totale due aux corps lamellaires et au matériel autre que les corps lamellaires, et l'estimation de l'absorption due au matériel non sédimentable, autre que les corps lamellaires. On a utilisé ces valeurs pour créer deux nouveaux paramètres: ΔA ($A_{250} - A_{10000}$) pour mieux estimer l'absorption causée par les corps lamellaires, et %A ($\Delta A/A_{250} \times 100$) pour exprimer l'absorption due aux corps lamellaires en terme d'absorption observable totale et ainsi minimiser les effets de la dilution. On a utilisé les trois paramètres (A_{250}, ΔA, %A) en combinaison pour créer une batterie (A_{Batt}) de valeurs d'absorption pour chaque échantillon de liquide. On a aussi évalué à des fins de comparaison l'absorption après centrifugation à 2 000 × g pendant 10 minutes (A_{2000}), méthode standard largement utilisée. A_{250} est désigné comme mature lorsqu'il est supérieur ou égal à 0,350, ΔA lorsqu'il est supérieur ou égal à 0,250, et %A lorsqu'il est supérieur ou égal à 75%. Si l'un de ces paramètres est immature, A_{Batt} est dit immature. La gamme des valeurs de A_{250}, A_{2000}, et A_{10000} (figure 1) augmente avec l'âge gestationnel aussi bien dans la population totale que dans la population corrigée (excluant les contaminants du liquide amniotique, et les grossesses avec isoimmunisation ou diabétiques). Les valeurs médianes de A_{250} augmentent plus que celles d'A_{2000} pour les âges gestationnels avancés. L'absorption résiduelle, non sédimentable (A_{10000}) augmente aussi pour les âges gestationnels avancés, toutefois sa contribution relative à A_{250} est moindre que celle à A_{2000} au-delà de 36 semaines. La seconde partie de l'étude a été d'évaluer la relation entre les valeurs de L/S, les paramètres d'absorption et les complications chez le nouveau-né l'accouchement s'est produit dans les 3 jours suivant le prélèvement de liquide amniotique. L'absence de survenue de MMH est prévue le plus précisément par le rapport L/S dans la population totale (tableau II), et également par les 3 méthodes dans la population corrigée. Les fausses prévisions de maturité sur la base des données de l'absorption ont été éliminées quand la population étudiée était limitée aux specimens non contaminés obtenus en l'absence de diabète maternel, d'isoimmunisation et de rupture prématurée des membranes.

Cette étude démontre que l'absence de survenue de la MMH peut être prévue également par le rapport L/S, le A_{Batt} ou la méthode du A_{2000}. Ces données suggèrent que l'utilité du A_{Batt} provient principalement de la teneur du liquide amniotique en corps lamellaires, alors que, et les corps lamellaires, et le matériel non sédimentable contribuent au A_{2000}.

Mots-clés: Absorption à 650 nm, analyse du liquide amniotique, maturité pulmonaire fœtale.

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